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Biosynthesis of Propolis-silver nanoparticles: Optimization and In Vitro Validation for antimicrobial applications

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Abstract: There is a current research focus on producing bioactive nanomaterials through eco-friendly approaches because of the wide range of uses for these materials in different industries. In this study, a simple and environmentally conscious chemistry approach was utilized to produce silver nanoparticles (AgNPs) using Egyptian propolis as the reducing and capping agent. The resulting silver nanoparticles were characterized using several methods, including UV spectroscopy and TEM. The dimensions of the synthesized silver nanoparticles measured 4.5 ± 1 nm, increasing to 11 ± 4 nm with higher concentrations of silver nitrate. The antimicrobial properties of PP samples, enhanced by varying concentrations of Ag-NPs, effectively inhibit *Escherichia coli, Streptococcus mutans*, and *Candida albicans*. Moreover, high MIC values were observed among all samples against *Escherichia coli*. The antimicrobial activity increases with the increase of Ag-NPs concentration in the PP extracts. These results demonstrate the potential of the Ag-PP sample as an effective antimicrobial agent to combat against bacterial infection.

Keywords: silver nanoparticles, propolis, antimicrobial activity, biomedical application.

1. Introduction

Propolis is a natural, hydrophobic substance produced by honeybees, consisting of plant secretions. The unique composition of propolis can vary significantly based on the plants the bees interact with, as well as geographical and temporal factors. Typically, propolis consists of approximately 50% resin, 30% wax, 10% essential oil, 5% pollen, and 5% other substances. Egyptian propolis has a complicated chemical composition with only a few recognized groups. Egyptian propolis was found to be mostly composed of a mixture of flavonoids and phenolic acids, aromatic acids, and related esters, aldehydes, and ketones. This resin is considered the polyphenolic component. Additionally, propolis includes foam, essential oils, pollen, and other organic substances such as amino acids, steroids, alcohol, polysaccharides, hydrocarbons, hydroxybenzenes, and water[1-3]. Propolis is an exceptional natural biomaterial that offers a range of beneficial properties, making it highly preferred for wound healing applications. Known for its antimicrobial, antioxidant, and anti-inflammatory characteristics, propolis has been utilized in traditional and complementary medicine since the time of the Ancient Egyptians due to its innate antiseptic and anesthetic qualities. [4, 5]. Topical application of propolis stimulates the accumulation of sulfated glycosaminoglycans, which are essential for tissue granulation in the wound bed, tissue growth, and wound closure. [6]. Nanomaterials play a vital role in the advancement of potentially sustainable technologies for humanity. The synthesis of nanoparticles through green chemistry methods utilizing biomaterials bridges nanotechnology with biotechnology. This area of chemistry, known as green chemistry, employs biomaterials for the bio-reduction of metal ions into their elemental form within the size range

of 1–100 nm, referred to as green nanoparticle synthesis. The green synthesis process is more dependable, quicker, and cost-effective, and it can be readily scaled up for larger operations.[7-9].

Nanoparticles composed of metals such as silver (AgNPs), gold (AuNPs), zinc oxide (ZnONPs), titanium oxide (TiO2NPs), and copper oxide (CuONPs) have demonstrated significant and lasting antimicrobial properties against a variety of bacteria, fungi, algae, and even viruses [10-13]. These metal-based nanoparticles utilize multiple bactericidal mechanisms, including the induction of oxidative stress, interaction with bacterial biomolecules (resulting in damage to cell membranes or cellular organelles), and the use of non-specific strategies (such as disrupting cell signaling processes).14,15] These aspects underscore the considerable promise of metal-based nanoparticles at the nanoscale as viable alternative antibacterial agents to tackle antibiotic resistance in bacteria. Research and advancements in nanotechnology, particularly concerning the well-known nanoproduct—nanosilver—have proven its outstanding antibacterial and antiviral properties.[16] Nanosilver particles (AgNPs), which are smaller than 100 nm and consist of 20 to 15,000 silver atoms, can degrade bacterial, viral, and fungal cells. This effect mirrors that of antibiotics, with the key difference being that pathogens do not appear to develop resistance to these nanoparticles[17,18].

Propolis serves as both a reducing and stabilizing agent in the environmentally friendly synthesis of silver nanoparticles (AgNPs) because of its abundant array of bioactive compounds, which include flavonoids, phenolic acids, terpenes, and various polyphenols. Polyphenols, flavonoids, and various reducing agents present in propolis transfer electrons to Ag^{+} ions, transforming them into elemental silver (Ag^{0}). Compounds such as caffeic acid and quercetin contain hydroxyl (-OH) groups that function as electron donors. The reduced silver atoms (Ag^{0}) begin to nucleate, forming tiny clusters. These clusters amalgamate and develop into nanoparticles as they aggregate and stabilize[19-21]. Recent study explored the biosynthesis of silver nanoparticles (AgNPs) using Sonoran Desert propolis, focusing on their effectiveness against multi-drug resistant (MDR) bacteria. The study revealed that AgNPs synthesized with winter-collected propolis were especially effective, with an average nanoparticle size around 16.5 nm. These nanoparticles displayed significant antibacterial and antibiofilm activities against MDR strains of *E. coli* and *Staphylococcus aureus*, indicating potential applications for treating infections where antibiotic resistance is a challenge. The research also confirmed that these propolis-mediated AgNPs were non-toxic to human cell lines like HeLa and ARPE-19, suggesting a safe profile for biomedical applications[21]. In addition, propolis-AgNP surgical sutures offer an eco-friendly and potentially safer alternative to synthetic antimicrobial sutures, showing promise for preventing surgical site infections and promoting wound healing in clinical settings[22, 23]. The microstructure and biological studies have demonstrated that these propolis-AgNP-coated sutures possess strong antimicrobial properties, significantly inhibiting the growth of common wound pathogens like *Staphylococcus aureus* and *Escherichia coli*. Furthermore, in vitro cell culture studies with fibroblast cells have shown that these sutures are biocompatible, supporting cellular viability and proliferation, which is crucial for wound healing applications. Wound healing assays indicate that sutures coated with propolis and silver nanoparticles (AgNP) may enhance cell migration and tissue regeneration, which are crucial for effective wound healing and preventing infections in surgical environments. Propolis combined with silver nanoparticles offers a flexible and efficient alternative for antimicrobial and antioxidant applications across various fields.[22] However, it is essential to conduct thorough evaluations of their combined effects in living organisms. Specifically, silver nanoparticles may pose risks to living cells; therefore, controlled usage and further studies on their health effects are necessary. Additionally, standardizing the concentration and size of the silver particles in combination with propolis can help reduce toxicity and ensure safety in biomedical applications.[24]

2. Materials and methods

2.1 Materials

Silver nitrate (AgNO3, ≥99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Propolis Samples and Extract Preparation

Seasonal samples of propolis were collected in winter 2023 from Mansoura, dakhali, Egypt by a local beekeeper. For the extraction, 20 g of the collected propolis were added to 200 mL of ethanol /methanol mixture solution (50:50 v/v) in a closed Erlenmeyer flask and kept at 40 ^{o}C away from light. After three days, the suspension was filtered using Whatman® cellulose filter papers, grdae 1. Next, ethanol/methanol solution was evaporated under reduced pressure, to obtain the propolis extract. Samples were stored at −20 °C.

Synthesis of Silver Nanoparticles

For the synthesis of AgNPs, a Stock solution of propolis were prepared in ethanol solution (50 mg/mL). The obtained PP stock solution was added to 10 mL aqueous solution of silver nitrate ($AgNO_3$, 1 mM or 0.5 mM) solution. The mixture pH was adjusted at 9 using 0.1 M NaOH. The $AgNO_3$/PP suspension was maintained under continuous stirring in a magnetic stirrer for 2 h at 1000 rpm at room temperature. During this step, the light brownish color solution changed into dark brown color which indicates the onset of formation AgNPs. The suspension was incubated overnight in a shaking water bath in dark for the synthesis of PP-AgNPs. After that, the obtained PP-AgNPs suspensions were centrifuged at 10,000× g for 10 min and washed twice with Milli-Q water. Finally, the wet PP-AgNPs samples were lypholozed at -54^{o}C for further use and characterization.

Preparation of Electrospun PP andPP-AgNPs/PCL composite mats

In this study, various PCL mats were produced through electrospinning a 10 wt.% PCL solution in chloroform that included 10 wt.% PP and 10 wt.% PP-AgNPs relative to the PCL content. To prepare the 10 wt.% PCL solution, PCL pellets were dissolved in chloroform, followed by the gradual addition of PP and PP-AgNPs while maintaining continuous magnetic stirring. The electrospinning process occurred at room temperature, with parameters set to 20 kV for the applied voltage, a tip-to-collector distance of 14 cm, and a solution feed rate of 0.5 ml/h. Electrospun fibers were collected on the surface of a rotating plate (10 cm in diameter) using an aluminum foil sheet. Following the electrospinning, the nanofibrous mat was placed in a vacuum for 12 hours at 25 °C to dry.

Microstructure Characterizations

Transmission electron microscopy (TEM):- The sample powder was dispersed and dropped on a copper grid with carbon film supported. The samples were observed through TEM (JEM-2100F, JEOL Ltd., Japan).

Antimicrobial activity

Antimicrobial effectiveness of the samples that were prepared was evaluated against three distinct microorganisms, including gram-negative bacteria *(Escherichia coli: ATCC25922*), gram-positive bacteria (*S. mutans: ATCC6633*), and unicellular fungi (*Candida albicans: ATCC90028*). The bacterial strains were cultivated on nutrient agar at a temperature of 37 °C for a duration of 24 hours, whereas the fungal strains were inoculated onto malt extract agar (MEA) plates, incubated for three days at 28 ± 2 °C, and subsequently stored at 4 °C for later use. The minimal inhibitory concentration (MIC) for the PP-AgNPs colloidal solution was established following the M07-A9 protocol set forth by the Clinical Laboratory Standards Institute [21]. The silver-containing solutions that were prepared were diluted to various concentrations ranging from 1000 to 10 µg/mL, and each one was evaluated separately to identify the MIC against the chosen bacterial strains (with Amoxicillin Clavulanate (AMC) serving as the benchmark antibiotic) and fungal strains (using nystatin as the standard antifungal agent [22]). The antibacterial activity of the prepared colloidal and cotton fabric samples was examined using the agar well diffusion method to measure the inhibition zone of the tested samples against different isolated bacterial and fungal pathogens [24].

3. Results and Discussion

In this study, the reduction capacity of PP was determined to prepare AgNPs under mild condition pH 8.5 and at room temperature (24 °C), in which different concentration of silver nitrate precursor (50 mM or 100 mM) were added to PP (50 mg/mL). The PP-AgNPs were collected after 3 h of reaction time in dark and characterized by UV-Vis spectrophotometry, from a range of 240–800 nm. As shown in Figure 1A, an absorption peak was appeared at about 428 nm (50 mM $AgNO_3$) and 436nm (100 mM $AgNO_3$), indicating the formation of AgNPs. In contrast, upon increasing silver precursor concentration, more AgNPs were formed with large diameter, especially at 1 mM of $AgNO_3$, as observed by the increase of intensity of absorption peak at 430 of the localized surface plasmon resonance (LSPR) (**Figure 1**). In addition, an absorption peak was observed around 260 nm related to the PP extract (black arrows).

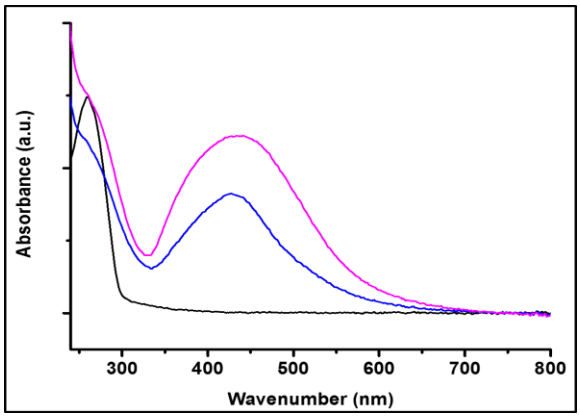

Fig.1 shows (A) UV-Vis spectra of PP-AgNPs at different concentration of silver precursor.

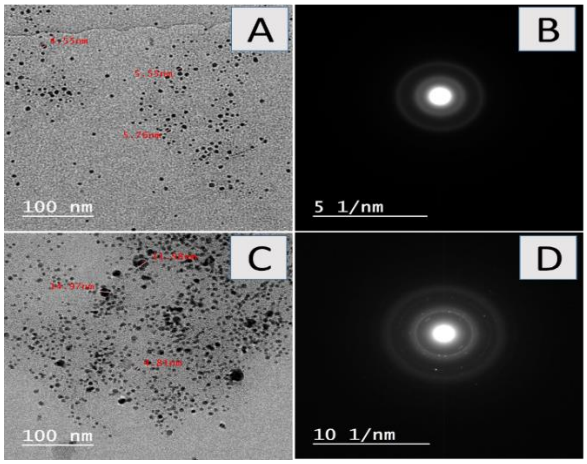

Figure 2 represents TEM micrographs of the as-prepared PP-AgNPs colloidal solutions: (A,B) PP-AgNPs (50 mM) and (C&D) PP-AgNPs (100 mM) .

The TEM results showed that the diameters of the AgNPs varied across the different samples, as illustrated in **Figure 2**. The average diameter of the PP-AgNPs at 50 mM was approximately 4.5 ± 1 nm, which increased to 11 ± 4 nm for the PP-AgNPs at 100 mM. The inserted SAED images demonstrate a diffraction pattern that indicates the crystalline structure of the synthesized AgNPs.

Assessment of antimicrobial activity

Propolis is a resinous material extracted from the buds and flowers of plants and used as a protective agent against microbial contamination. Polyphenols are the major chemical components of PP, and they are responsible for its antimicrobial activity. The antimicrobial action of PP and PP-AgNPs samples was explored on pathogens for humans using different approaches [22-24]. The antimicrobial properties of PP are illustrated in **Table 1** and **Figure 3**. While PP demonstrated antimicrobial capabilities against all examined microorganisms, the PP-AgNPs samples displayed more potent effects against these organisms. The findings reveal a notably greater antimicrobial activity against *C. albicans* and *S. mutans*, although the effectiveness was lower against *E. coli*. Additionally, the Minimum Inhibitory Concentration (MIC) was assessed by evaluating antimicrobial activity at various concentrations.

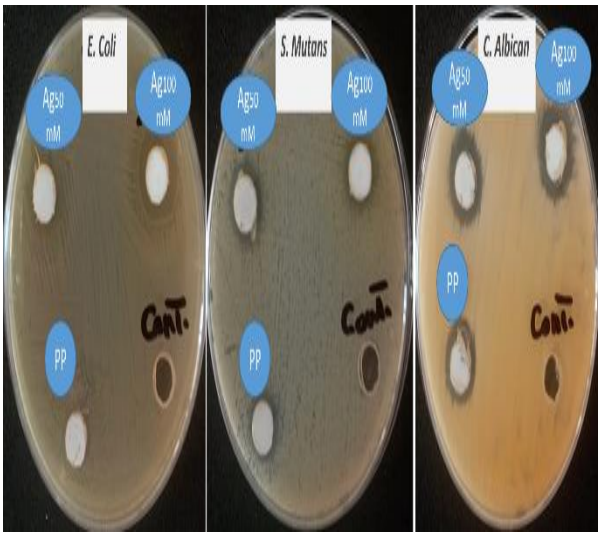

Fig.3 shows the antimicrobial effectiveness, indicated by halo-zones of the PP at various concentrations, was assessed using the agar well diffusion method against three pathogenic microorganisms.

Table-1 MIC values calculated at experimental points.

Sample Name	Minimal Inhibitory Concentration (MIC) µg/mL		
	E. coli	S. mutans	C. albicans
PP	243 ±34.91	180 ±14.17	123.8 ± 29.65
PP-AgNPs (50mM)	166 ± 27.1	91.93 ± 18.91	89.5 ± 16.23
PP-AgNPs (100mM)	167.43 ±25.6	68.93 ± 15.2	80.19 ± 3.2

Conclusion

Colloidal solutions of silver nanoparticles with high stability were effectively created in an eco-friendly manner through the direct reduction of silver nitrate using PP extract. TEM analysis revealed that the size of AgNP particles synthesized from PP samples varied based on the concentration of Ag ions, ranging from 4.5 ± 1 nm to 11 ± 4 nm. In vitro testing confirmed that propolis-synthesized silver nanoparticles (AgNPs) exhibit broad-spectrum antibacterial activity, validating their potential for wound care applications, where bacterial infections pose a significant clinical challenge.

4. References

1. Parolia, A., et al., (2010) Propolis and its potential uses in oral health.. **2**(7): p. 210-215.
2. Miguel, M.G., M.D.J.J.o.p. (2011) Antunes, and b. sciences, Is propolis safe as an alternative medicine?. **3**(4): p. 479-495.
3. Castaldo, S. and F.J.F. Capasso, (2002) Propolis, an old remedy used in modern medicine.. **73**: p. S1-S6.
4. Kasote, D., V. Bankova, and A.M.J.P.R. Viljoen, (2022) Propolis: Chemical diversity and challenges in quality control.. **21**(6): p. 1887-1911.
5. Zullkiflee, N., H. Taha, and A.J.M. Usman, (2022) Propolis: Its role and efficacy in human health and diseases.. **27**(18): p. 6120.
6. Lesmana, R., et al., (2022) The suitability of propolis as a bioactive component of biomaterials.. **13**: p. 930515.
7. Dumitru, C.D., et al., (2022) Bee-derived products: chemical composition and applications in skin tissue engineering.. **14**(4): p. 750.
8. Oršolić, N.J.M., (2022) Allergic inflammation: Effect of propolis and its flavonoids.. **27**(19): p. 6694.
9. Salatino, A.J.M., (2022) Perspectives for uses of propolis in therapy against infectious diseases.. **27**(14): p. 4594.
10. Patil, K.B., et al., (2020) Metal based Nanomaterial's (Silver and Gold): Synthesis and Biomedical application.. **10**(2): p. 97-106.
11. Sánchez-López, E., et al., (2020) Metal-based nanoparticles as antimicrobial agents: an overview.. **10**(2): p. 292.
12. Luo, Y.-H., L.W. Chang, and P.J.B.r.i. Lin, (2015) Metal-based nanoparticles and the immune system: activation, inflammation, and potential applications.. **2015**(1): p. 143720.
13. Klębowski, B., et al., (2018) Applications of noble metal-based nanoparticles in medicine.. **19**(12): p. 4031.
14. Skłodowski, K., et al., (2023) Metallic nanosystems in the development of antimicrobial strategies with high antimicrobial activity and high biocompatibility.. **24**(3): p. 2104.
15. Aboelmagd, L.A., E. Tolba, and Z.A.J.P.B. AbdelAziz, (2023) Chitosan–organosilica hybrid decorated with silver nanoparticles for antimicrobial wearable cotton fabrics.. **80**(4): p. 4229-4243.
16. Mukherji, S., et al., (2019) Synthesis and characterization of size-and shape-controlled silver nanoparticles.. **4**(1): p. 20170082.
17. Pryshchepa, O., et al., (2020) Silver nanoparticles: Synthesis, investigation techniques, and properties.. **284**: p. 102246.
18. Hammed, V., et al., (2024) Silver nanoparticles (AGNPs): A review on properties and behavior of silver at the nanoscale level.. **12**(2): p. 1267-1272.
19. Ghramh, H.A., et al., (2019) Biogenic synthesis of silver nanoparticles using propolis extract, their characterization, and biological activities.. **11**(6): p. 876-883.
20. Matei, P.M., et al., (2015) Synthesis of chitosan oligomers/propolis/silver nanoparticles composite systems and

study of their activity against Diplodia seriata.. **2015**(1): p. 864729.
21. Mendez-Pfeiffer, P., et al., (2022) Biosynthesis of Silver Nanoparticles Using Seasonal Samples of Sonoran Desert Propolis: Evaluation of Its Antibacterial Activity against Clinical Isolates of Multi-Drug Resistant Bacteria.. **14**(9): p. 1853.
22. Selvaraju, G.D., et al., (2022) Fabrication and characterization of surgical sutures with propolis silver nano particles and analysis of its antimicrobial properties.. **34**(5): p. 102082.
23. Baygar,T.J.T.J.o.M.S., (2020) Characterization of silk sutures coated with propolis and biogenic silver nanoparticles (AgNPs); an eco-friendly solution with wound healing potential against surgical site infections (SSIs).. **50**(1): p. 258-266.
24. Miguel, S.P., et al., (2019) An overview of electrospun membranes loaded with bioactive molecules for improving the wound healing process.. **139**: p. 1-22.